View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by University of Northern Iowa

Proceedings of the Iowa Academy of Science

Volume 82 | Number 3-4 Article 9

1976

Nitrogen Metabolism in Acanthamoeba castellanii: Amino Acid Consumption and Composition Patterns

Warren D. Dolphin
Iowa State University

Let us know how access to this document benefits you

Copyright ©1976 Iowa Academy of Science, Inc.

Follow this and additional works at: https://scholarworks.uni.edu/pias

Recommended Citation

Dolphin, Warren D. (1976) "Nitrogen Metabolism in Acanthamoeba castellanii: Amino Acid Consumption and Composition Patterns," *Proceedings of the Iowa Academy of Science, 82(3-4),* 171-174.
Available at: https://scholarworks.uni.edu/pias/vol82/iss3/9

This Research is brought to you for free and open access by the Iowa Academy of Science at UNI ScholarWorks. It has been accepted for inclusion in Proceedings of the Iowa Academy of Science by an authorized editor of UNI ScholarWorks. For more information, please contact scholarworks@uni.edu.

Nitrogen Metabolism in *Acanthamoeba castellanii:* Amino Acid Consumption and Composition Patterns

WARREN D. DOLPHIN[1]

DOLPHIN, WARREN D. (Department of Zoology, Iowa State University, Ames, Iowa 50011). Nitrogen Metabolism in *Acanthamoeba castellanii:* Amino Acid Consumption and Composition Patterns. *Proc. Iowa Acad. Sci.* 82(3-4): 171-174, 1976.

Quantitative analyses of the amino acids in the culture media before and after population growth demonstrate that *Acanthamoeba castellanii* selectively utilizes amino acids from Adam's defined medium (DM_2) and Neff's optimal growth medium, but no amino acid is completely depleted during normal population growth. The cellular amino acid content varies for amoebae grown in the two media. The most common total cellular amino acids are alanine, aspartic acid, glutamic acid, glycine, and leucine. The most common free amino acids in the intracellular pools are alanine and proline. In DM_2, NH_3 is produced at a rate of 0.027 picomoles/amoebae/hour.

INDEX DESCRIPTORS: Amino Acids, Nutrition, *Acanthamoeba.*

The most recent reviews of protozoan nutrition and physiology indicate a dearth of information regarding nitrogen metabolism in the protozoa except for a few well-studied species of flagellates and ciliates (Kidder, 1967; Wagtendonk and Soldo, 1970). Studies of nitrogen metabolism in the sarcodinids have dealt primarily with the question of nutritional requirements and macromolecular metabolism, neglecting the area of nitrogen compound interconversions. The Hartmannellid amoebae are no exception to this generalization, though they can be grown on strictly defined media (Adam, 1959, 1964; Adam and Blewett, 1967; Band, 1962), making them excellent experimental material to be used in studies of sarcodinid metabolism. In a series of experiments, of which this is the first, various aspects of nitrogen metabolism in *Acanthamoeba castellanii* will be examined with the ultimate goal of describing transport, metabolism, and excretion of nitrogenous materials.

This study of the amino acid nutrition of *Acanthamoeba* was undertaken to determine (1) what amino acids are utilized from Neff's optimal growth medium (OGM) and Adam's defined medium (DM_2), (2) the amoeba's amino acid composition in these media, and (3) the excretory products of amino acid catabolism.

MATERIALS AND METHODS

Acanthamoeba castellanii (Neff's I-12 strain) was axenically grown in OGM (Neff *et al.*, 1958) or DM_2 (Adam, 1964), except that in the latter the minor salt component was doubled. In both media, 100 mM glucose was the carbon source. The initial pH was adjusted to 5.5 in DM_2 and 6.5 in OGM.

For the amino acid utilization studies, amoebae were cultured in sterile, capped centrifuge tubes. When the population had reached maximum density, the tubes were gently centrifuged, and the growth medium transferred to another sterile tube and reinoculated with more amoebae. This procedure was repeated four times to ensure detectable reductions in amino acid levels in the media. After the last transfer, the culture medium was harvested for amino acid analysis.

To measure free cellular and protein amino acids, the amoebae were removed from the growth medium and washed with 0.15 M KCl in 5% sucrose. The cells were left in the washing medium for one hour before being fractionated to allow pinocytotic vacuoles containing growth medium to be metabolized. Free cellular amino acids were separated from proteins by treating the amoebae with 10% tricholacetic acid (TCA). The TCA-precipitated proteins were washed with 95% ethanol and hydrolyzed with 6 N HCl for 22 hours. The TCA-soluble material was extracted three times with diethyl ether to remove the TCA, and the resultant solution lyophilized.

Amino acid analyses of culture media and cell fractions were done on a Beckman 120B Analyser using "hydrolysate columns." The detection limit is estimated to be 10^{-9} moles per ml.

Ammonia production was assayed in Warburg flasks containing a cell suspension in growth medium and filter paper saturated with 0.5 N H_2SO_4 in the center well. The stoppered flasks were incubated with agitation at 30°C and the reactions stopped by tilting 2N NaOH into the flask from the side arm. This raised the pH to 12, and quantitatively transferred NH_3 to the center well in three hours. The center well contents, after being neutralized with NaOH, were analyzed for the presence of NH_3 by the salicylate-dichloroiso cyanurate method of Reardon *et al.* (1966).

RESULTS

Amino acid utilization was observed by sampling the amino acids in the two growth media initially and after four cycles of population growth (Tables 1 and 2). Both OGM samples were acid-hydrolyzed to digest any peptides that might be present and serve as amino acid sources when ingested pinocytotically by the amoebae. DM_2 samples were not acid-hydrolyzed. All amino acids assayed were consumed to some degree. Arginine, histidine, isoleucine, leucine, phenylalanine, threonine, and valine had the greatest changes in both media. The minimal amino acids required for growth by *Acanthamoeba castellanii,* Neff's strain, are considered to

[1] Department of Zoology, Iowa State University, Ames, Iowa, 50011.

TABLE 1. AMINO ACID UTILIZATION FROM DM_2 BY *Acanthameoba*

Amino Acid	*10^{-6} Moles/ml Fresh*	*Conditioned*	*Percent Decrease*
L-Alanine	*	0.04	
†L-Arginine	6.65	4.43	33.4
†Glycine	32.38	22.88	29.4
L-Histidine	4.88	3.25	33.6
†L-Isoleucine	6.56	4.52	31.1
†L-Leucine	6.30	4.35	31.0
L-Lysine	3.88	3.26	16.0
†L-Methionine	1.14	0.86	24.6
L-Phenylalanine	3.22	2.18	32.3
DL-Threonine	5.63	3.91	30.6
L-Tryptophan	1.23	1.14	7.3
†DL-Valine	5.05	3.69	26.9
Total $\propto$ NH_2 N	76.92	54.51	28.9 (Average)

* Below detection limit.
† Essential amino acids (2,3).

TABLE 2. AMINO ACID UTILIZATION FROM OGM BY *Acanthamoeba*

Amino Acid	*10^{-6} Moles/ml* Fresh*	*Conditioned*	*Percent Decrease*
Alanine	8.06	6.02	25.2
†Arginine	2.34	1.20	48.8
Aspartic Acid	6.58	3.64	44.6
Glutamic Acid	10.37	5.68	45.3
†Glycine	11.42	7.66	32.1
Histidine	1.10	0.56	49.1
†Isoleucine	3.10	1.78	42.6
†Leucine	4.80	2.56	46.7
Lysine	4.28	2.84	33.7
†Methionine	1.21	0.82	32.2
Phenylalanine	2.09	1.26	39.6
Proline	4.96	4.07	18.0
Serine	3.83	2.29	40.3
Threonine	3.22	1.90	41.0
Tryptophan	destroyed by acid hydrolysis		
Tyrosine	0.75	0.70	6.8
†Valine	4.33	2.33	46.2
Total $\propto$ NH_2 N	72.44	45.31	37.3 (Average)

* Entries are averages of two determinations.
† Essential amino acids (2,3).

be arginine, glycine, methionine, leucine, isoleucine, and valine (Adam, 1964; Adam and Blewett, 1967). These are among the preferred amino acids in both media, but they do not represent the complete group of highly used amino acids.

All amino acids contained in OGM are of biological origin and, presumably, L isomers but racemic (DL) mixtures of valine and threonine were used in formulating the DM_2. Assuming that *Acanthamoeba* lacks D amino acid oxidase (Muller and Moller, 1969) the percent decrease for the L form is greater than that listed in Table 3. Aspartic acid, glutamic acid, and serine, which occur only in OGM, are used extensively, probably reflecting their central role in synthetic functions. Alanine, lysine, proline, tryptophan, and tyrosine are not utilized to the same extent as the other amino acids. A general conclusion drawn from these tables is that there appears to be some selectivity in the utilization of amino acids by *Acanthamoeba*. Whether these differences represent selective uptake or differential release from the amoebae after pinocytotic ingestion of the medium is yet to be determined.

The amino acid composition of the amoebae in the two growth media is given in Tables 3 and 4. The major amino acids of *Acanthamoeba* are alanine, aspartate, glutamate, glycine, and leucine, which collectively represent 49% of the amino acid molecules in amoebae grown in OGM or 44% from DM_2. Alanine and proline are the common free intracellular amino acids. The determination of free cellular amino acids may have been influenced by the occurrence of pinocytotic vacuoles. Despite precautions that were taken to avoid this, the values reported may be somewhat higher than the actual pool values.

Utilization of amino acids in other than protein synthesis should result in nitrogenous excretory products such as NH_3 (Cailleau, 1934; Neff *et al.*, 1958). Kinetic studies of NH_3 production of *Acanthamoeba* in DM_2 and OGM indicated linear NH_3 production at a rate of 0.027 picomoles per amoeba per hour. Amoebae grown in DM_2 have less protein content than amoebae in OGM and, therefore, a higher rate of deamination per mg of protein. Ammonia, however, is not the only nitrogenous material produced. Purines and pyrimidines have also been found in DM_2 and OGM after growth. Since OGM initially contains nitrogen bases in various forms and DM_2 lacks these bases, an interesting comparative system is available for future work. In DM_2, alanine was found in small amounts in conditioned medium (Table 1). It may be a true excretory product or may merely represent loss from intracellular pools (Table 3) through cell breakage.

DISCUSSION

There appears to be a differential utilization of the amino acids in the growth medium. Bowers and Olszewski (1972) have suggested that *Acanthamoeba* feeds pinocytotically in a non-selective fashion, though they did not rule out the existence of specific transport mechanisms. If amino acids are transported primarly by food vacuoles, then the differences in utilization rates observed are the result of differential loss and not uptake. Bowers and Olszewski demonstrated that leucine (as well as other molecules) was ingested at a rate corresponding to 2 μl of medium/hour/10^6 amoebae. If this rate of pinocytosis is used to calculate the time necessary to pump 31% of the DM_2 through the average number of amoebae present in one of the cultures in these experiments (estimated to be 3-4 X 10^5 amoebae/ml), the time obtained (388-517 hours) is in fair agreement with the age of the cultures. A similar calculation can be made for 47% utilization from OGM. This, along with the relatively low utilization rates and the inability to grow in a diluted medium (Adam, 1964), leads to the speculation that the selectivity observed is in leakage from the cell and not transport into the amoebae.

The amino acid composition of *Acanthamoeba* is in general agreement with the previous qualitative report of Schleicher (1959) for amoebae proteins and the quantitative report by Drainville and Gagnon (1973) for amino acid pools. The latter authors found that 60-80% of the total amino acid pool consisted of alanine, proline, and γ-amino butyric acid (GABA). In this study, alanine and proline made up 68% of the intracellular pool of amino acids in OGM-grown amoebae, and 60% in DM_2.

These high pool levels correlate positively with the observations of Husain and Mohan Rao (1969) and Borut (1959) on the active types of transaminases in Hartmannellids. In

Dolphin: Nitrogen Metabolism in Acanthamoeba castellanii: Amino Acid Consu

TABLE 3. AMINO ACID COMPOSITION OF *Acanthamoeba* GROWN IN DM_2

		Protein Amino Acids			Free Amino Acids	
Amino Acid	*Total* 10^{-14} *M/Cell*	*nM/mg Protein*	10^{-14} *M/Cell*	*Percent Total*	10^{-14} *M/Cell*	*Percent Total*
Alanine	4.80	.487	3.37	70.2	1.430	29.8
†Arginine	1.90	.271	1.88	99.0	.016	1.0
Aspartic Acid	3.29	.467	3.24	98.6	.048	1.4
Half Cystine	0.03**	*	*		.027	
Glutamic Acid	4.22	.557	3.86	91.4	.361	8.6
†Glycine	3.37	.413	2.86	84.8	.511	15.2
Histidine	0.88	.104	0.72	82.0	.159	18.0
†Isoleucine	1.60	.228	1.58	98.8	.024	1.2
†Leucine	2.86	.408	2.83	99.0	.027	1.0
Lysine	2.68	.379	2.63	98.0	.047	2.0
†Methionine	0.71**	.102	0.71		*	*
Phenylalanine	1.31	.187	1.30	99.4	.012	0.6
Proline	2.38	.250	1.73	72.8	.649	27.2
Serine	2.18	.281	1.95	89.4	.230	10.6
Threonine	1.98	.276	1.91	96.3	.067	3.7
Tyrosine	1.04	.146	1.01	97.2	.029	2.8
†Valine	2.51	.342	2.37	94.5	.136	5.5

† Essential amino acids (2,3).
* Below detection limit.
** Approximation.

TABLE 4. AMINO ACID COMPOSITION OF *Acanthamoeba* GROWN IN OGM

		Protein Amino Acids			Free Amino Acids	
Amino Acid	*Total* 10^{-14} *M/Cell*	*nM/mg Protein*	10^{-14} *M/Cell*	*Percent Total*	10^{-14} *M/Cell*	*Percent Total*
Alanine	27.7	0.525	25.3	93	2.420	7.0
†Arginine	12.5	0.260	12.5	100	0.035	
Aspartic Acid	25.6	0.527	25.4	99.2	0.201	0.8
Half Cystine	0.1	*			0.071	
Glutamic Acid	29.5	0.605	29.2	99.1	0.272	0.9
†Glycine	24.2	0.443	24.0	98.9	0.229	1.1
Histidine	5.0**	0.103	5.0			
†Isoleucine	12.1	0.248	12.0	99.6	0.051	0.4
†Leucine	23.0	0.473	22.8	99.3	0.154	0.7
Lysine	16.3	0.339	16.3			
†Methionine	5.1	0.106	5.1			
Phenylalanine	10.4**	0.216	10.4	100	Trace	
Proline	15.5	0.295	14.2	91.7	1.280	8.3
Serine	15.7	0.321	15.5	98.5	0.236	1.5
Threonine	15.0	0.307	14.8	98.9	0.173	1.1
Tryptophan		destroyed by acid hydrolysis				
Tyrosine	8.0	0.162	7.8	98.1	0.154	1.9
†Valine	16.1	0.332	16.0	99.2	0.130	0.8

† Essential amino acids (2,3).
* Below detection limit.
** Approximation.

Hartmannella, alanine was the most active amino donor of the 20 amino acids tested, followed by aspartic acid and serine. In *Mayorella,* aspartic acid and alanine were active donors, and strong glutamic acid decarboxylase activity leading to the formation of GABA was observed.

The function of the high pool level of proline may be osmoregulatory. Drainville and Gagnon (1973) demonstrated that increasing the osmolality of the growth medium led to increased intracellular levels of free amino acids. Proline pools showed the greatest increases, followed in order by hydroxyproline, alanine, serine, aspartic acid, and GABA.

Comparative data on free amino acids are available for other species of amoebae (Friz, 1968). Friz found that valine was the most common amino acid in *Pelomyxa carolinensis,* valine and an unknown in *Amoeba dubia,* and histidine in *A. proteus* when all three were grown under identical conditions. If the culture conditions were varied for *P. carolinensis,* valine remained a primary constituent of the intracellular pools, but alanine and glycine increased significantly. In *Acanthamoeba,* this shift in pool patterns was not seen. Alanine and proline remain the predominant intracellular pool constitutents in both of the media.

If the cellular protein amino acid concentrations are expressed as a molar ratio relative to glutamic acid, a remarkable constancy is observed. Most of the ratios for protein amino acids vary only a few percent between the two media. The lysine to glutamic acid ratio increases 18% in the protein of DM_2 amoebae compared to those from OGM. Argin-

Published by UNI ScholarWorks, 1975

ine, histidine, and valine show an average relative increase of 14%. Glycine decreases by 15% in DM_2-derived proteins compared to OGM. All other molar ratios change less than 10%.

Friz (1970) observed that *Amoeba proteus* does not show significant variations in protein amino acid patterns after starvation for 12 days and concluded that these patterns are independent of culture conditions. My observations suggest that some protein amino acid pattern variation is possible but there are not extensive protein composition changes associated with nutrition. These ratios might then serve as a useful taxonomic character for separating species.

To summarize, *Acanthamoeba* (1) selectively uses amino acids supplied in the growth medium, (2) does not deplete the amino acids in the growth medium during normal population growth, (3) produces NH_3 as a nitrogenous excretory product, (4) shows variations in pool levels of free amino acids in different growth media, and (5) has slight differences in its protein amino acid patterns related to type of culture media used.

ACKNOWLEDGMENT

This work was partially supported by funds from the Research Corporation and the Iowa State University Research Foundation. The assistance of Susan Paydon in maintaining the cultures for these experiments is gratefully acknowledged.

LITERATURE CITED

ADAM, K. 1959. The growth of *Acanthamoeba* sp. in a chemically defined medium. *J. Gen. Microbiol.* 21:219-229.

———. 1964. The amino acid requirements of *Acanthamoeba* sp. Neff. *J. Protozool.* 11:98-100.

——— and D. BLEWETT. 1967. Carbohydrate utilization by the soil amoeba *Hartmannella castellanii. J. Protozool.* 14:277-282.

BAND, R. 1962. The amino acid requirements of the soil amoeba, *Hartmannella rhysodes. J. Protozool.* 9:377-379.

BORUT, A. 1959. Studies on the oxidative metabolism of the amoeba *Mayorella palestinensis* Reich. Ph.D. Thesis, Hebrew University of Jerusalem.

BOWERS, B., and T. OLSZEWSKI. 1972. Pinocytosis in *Acanthamoeba castellanii:* Kinetics and morphology. *J. Cell Biol.* 53:681-694.

CAILLEAU, R. 1934. Utilisation des milieux liquides par *Acanthamoeba castellanii. Compt. Rend. Soc. Biol. (Paris)* 116:721-723.

DRAINVILLE, G., and A. GAGNON. 1973. Osmoregulation in *Acanthamoeba castellanii*—I. Variations of the concentrations of free intracellular amino acids and of the water content. *Comp. Biochem. Physiol.* 45A:379-388.

FRIZ, C. 1968. The free amino acid levels of *Pelomyxa carolinensis, Amoeba dubia* and *A. proteus. J. Protozool.* 15:149-152.

———. 1970. The protein amino acid compositions of *Amoeba proteus, A. discoides, A. dubia* and *Pelomyxa carolinensis. J. Protozool.* 17:235-239.

HUSAIN, M., and V. MOHAN RAO. 1969. Aminotransferase activity of *Hartmannella* (Culbertson strain A-1) grown axenically. *J. Gen. Microbiol.* 56:379-386.

KIDDER, G. 1967. Nitrogen: Distribution, Nutrition, and Metabolism. Pages 93-161 *in* Kidder, G. (ed.), *Chemical Zoology I.* Academic Press, New York.

MULLER, M., and K. MOLLER. 1969. Studies on some enzymes of purine metabolism in the amoebae *Chaos chaos* and *Amoeba proteus. Compt. Rend. Trav. Lab. Carlsberg* 36:463-497.

NEFF, R., R. NEFF, and R. TAYLOR. 1958. The nutrition and metabolism of a soil amoeba, *Acanthamoeba* sp. *Physiol. Zool.* 31:73-91.

REARDON, J., J. FOREMAN, and R. SEARCY. 1966. New reactants for the colorimetric determination of ammonia. *Clin. Chim. Acta* 14:403-405.

SCHLEICHER, J. 1959. Paper chromatography analysis of amino acids in Protozoa. Some aspects of the metabolism of aspartic acid. *Catholic Univ. Amer. Biol. Studies* 53:1-74.

WAGTENDONK, W. J. VAN, and A. SOLDO. 1970. Nitrogen Metabolism in Protozoa. Pages 1-56 *in* Campbell, J. (ed.), *Comparative Biochemistry of Nitrogen Metabolism I.* Academic Press, London.